Regional Rectal Perfusion: A New in Vivo Approach to Study Rectal Drug Absorption in Man

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Background: In vivo permeability measurements of drugs in the colonic/rectal region in humans are difficult. A new instrument for the perfusion of a defined and closed segment in the colon/rectum was developed. The objective of this study was to evaluate its use for studying drug absorption mechanisms in the human rectum and to investigate the effect of transmucosal water absorption on drug permeability. Six healthy subjects participated at 2 separate occasions by using a modified system for segmental rectal perfusion. The system consisted of a multichannel tube with inflatable balloons and was endoscopically introduced into the rectum. The technique was considered acceptable by the following criteria; (a) high and reproducible recovery of PEG 4000, (b) stable residence time of the solution within the test segment, (c) flux of electrolytes that agrees with previous reports, (d) mass-balance absorption of antipyrine across the rectal barrier, (e) and good acceptability to the subjects. The permeability of antipyrine in the rectal region was increased by inducing net water absorption. D-glucose was not absorbed during any study periods. The present technique is valuable for studying drug absorption from the human rectum.

KEY WORDS: intestinal permeability; rectal absorption; perfusion; bioavailability; human drug absorption.

INTRODUCTION

The importance of the rectum as a drug absorbing region has been emphasized by others, and it can offer a long residence time and low luminal peptidase activity (1). This might have some certain advantages for the absorption of drugs with reduced permeability across the epithelial barrier and/or increased sensitivity to enzymatic degradation in the upper part of the gastrointestinal tract (1). The effective permeability of drugs across the intestinal epithelium is influenced by several physico-chemical and physiological properties and may differ in various intestinal regions (2). Numerous studies using *in vitro* and *in situ* methods have been performed to predict the permeability and adsorption mechanisms of drugs (3-6). However, *in vivo* information on rectal permeability from humans are quite rare probably because of methodological problems, which are typical for open or semiopen colorectal perfusion systems (7, 8). Recently, a new instrument for the perfusion of defined and closed segments in the colon and rectum was developed (9). The system consists of a multichannel tube with inflatable balloons and is endoscopically introduced into the rectum and sigmoid colon. In order to obtain reliable and robust permability values by intestinal perfusion it is important to control the absorption condition and that proximal and/or distal luminal contents do not enter the test region, that the recovery of the perfusion fluid is high and stable over time, that the solution within the test segment is well-mixed, and that mass balance in absorption of drugs across the intestinal mucosa exists. Furthermore, the length of the segment has to be known to determine the absolute values of permeability and secretion flux. It is also important that the subjects respond positively to the use of the perfusion instrument.

A main purpose of this study was to evaluate the potential usefulness of the new colonic-rectal perfusion system for studying drug absorption mechanisms in the human rectum. We used antipyrine as a model drug, since it is unionised and highly soluble in water, devoid of potent pharmacological activity, and has a low metabolic extraction in the gut wall and liver (10-14). An other aim was to investigate how nutrients and electrolytes in the intestinal lumen and different osmotic pressure in luminal contents may influence drug permeability by affecting the net water flux across the rectal epithelium (15-17). The absorption of D-glucose and alterations in the concentrations of electrolytes (HCO_3^-, K^+, Na^+ and Cl^-) and osmolality were, therefore, determined in the outlet perfusion solution in parallel with absorption studies of antipyrine.

MATERIALS AND METHODS

Design and Positioning of the Tube

The perfusion was performed by using a modified system of a recently developed method for segmental colorectal perfusion (Fig. 1) (9). In this study, a tube made of polyvinyl chloride (PVC), with a total length of 40 cm and a inner and outer diameter of 10 and 16 mm, respectively, was used. Furthermore, the tube had three latex balloons attached to its wall. The two distal balloons surrounded the anal canal and functioned to stabilize the position and a third balloon to delimitate the rectal perfusion segment (Fig. 1). The tube consisted of five channels with an inner diameter of 1 mm each. Three of the channels were used to inflate the balloons and two were used for the perfusion of the rectal segment. The segment (8 cm long) was positioned in the mid-upper rectum with the aid of an endoscope (Olympus PQ 20). The positioning of the tube was preceded by an endoscopy of the rectum and sigmoid colon. With the endoscope in place, the tube (put on the endoscope) was slowly inserted into the intestinal lumen with the endoscope acting as guide. When the tube was positioned the two anal balloons were first inflated followed by inflation of the proximal segmental balloon. Next, the endoscope was withdrawn and the position of the tube in rectum was checked by fluoroscopy. The main channel of the tube functioned thereafter to decompress gas and fluids from the proximal bowel during the perfusion.

Study Design

Six healthy subjects (3 females and 3 males) all gave

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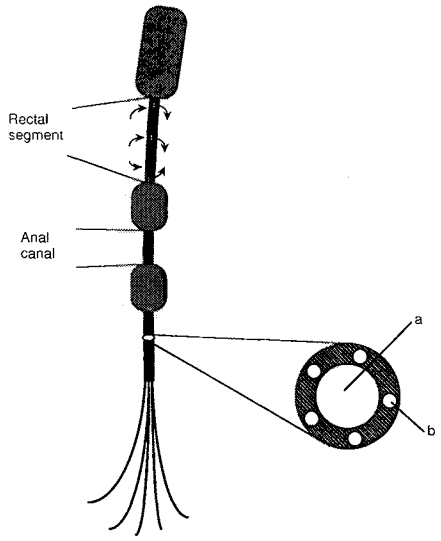

Fig. 1. A schematic presentation of the perfusion tube with 3 balloons delimiting the segment located in the mid-upper part of the rectum. Two balloons located on either side of the anal canal controlled the position of the tube. The central channel (a) was used for the introduction of the tube by the endoscope, and decompression of gas and fluids during perfusion. The smaller channels (b) were used to deliver air into the ballons and perfusion fluid to and from the rectal segment. The length of the perfused rectal segment is 8 cm.

informed consent to participate in the study, which was approved by the Ethics Committee of the Medical Faculty, Uppsala University. The subjects were prepared for routine flexible recto-sigmoid endoscopy, i.e., two days with diet restriction and an oral purgative (Pico-Salax, Ferring, Malmö, Sweden) in the morning and afternoon on the day before examination. In the morning, before intubation, a single dose of meperidine (25 mg) and diazepam (2.5 mg) were given intravenously as routine premedication.

The study was divided into two phases using perfusates of different composition. Each subject participated in two perfusion experiments on two separate occasions. An experiment lasted for 200 min and consisted of two periods of 100 min. The first period in each perfusion experiment (P1 and P3) was identical and served as a control. The perfusion experiment started by rinsing the segment with isotonic saline (37° C) for approximately 15-20 min, using a syringe pump (model 355, Sage Instrument, Orion Research Inc., Cambridge, MA). After the rinsing period, the perfusion of the segment started with the control solution for 100 min and thereafter the experimental solution for another 100 min. The perfusion rate was 3.0 ml/min. The perfusate leaving the rectal segment was collected quantively on ice in 10 min intervals throughout the perfusion. The syringes and perfusate were weighed and the samples were frozen immediately, being stored at −20° C until analysis. The subjects were recumbent during the 200 min perfusion period. After the cessation of the drug perfusion, the intestinal segment was rinsed with approximately 120 ml saline during 3-5 min to prevent further drug absorption. Blood samples were withdrawn from a cannula placed in a vein in the arm and collected in heparinized tubes at 0, 20, 40, 60, 80, 100, 120, 140, 160, 180, 200, 220, 240, and 300 min. The blood samples were centrifuged (2000 g for 10 min); the plasma was frozen immediately and stored at −20° C until analysis.

Drug and Perfusate Composition

Antipyrine (pKa = 1.5, K_D = 0.4, MW 188, K_D defined as octanol/water at pH 7.4) was supplied by Kabi-Pharmacia, Sweden. The concentration of the drug in the perfusion fluid that entered the intestinal segment was 2 mg/ml (10.5 mM) in both experiments. In the present study, one control solution (P1 and P3) and two other solutions differing in D-glucose concentration (P2) and osmolality (P4), were used. The isotonic control solutions (P1 and P3) consisted of 10 mM D-glucose, 5.4 mM KCl, 120mM NaCl, 2 mM Na_2HPO_4, 1 g/l polyethylene glycol (PEG 4000) MW 4000, and 35 mM mannitol. The osmolality was approximately 290 mosm/l. The first experimental solution (P2) was isotonic and almost identical to the control solution, except that it did not contain D-glucose. The second experimental solution was hypotonic (140mOsm/l) and consisted of 10 mM D-glucose, 20 mM KCl, 25 mM NaCl, 2 mM Na_2HPO_4, 1 g/l polyethylene glycol (PEG 4000) MW 4000, and 35 mM mannitol. Polyethylene glycol labeled with ^{14}C (^{14}C-PEG 4000) was purchased from Amersham Lab. Buckinghamshire, England and added to all three different perfusion solutions as a volume marker (2.5 μCi/l).

Stability and Adsorption Test of Antipyrine and D-Glucose

Incubation of antipyrine and D-glucose in the perfusion medium at 37°C for 180 min showed no degradation of the compounds. The stability of antipyrine and D-glucose in the intestinal perfusate was also tested. Luminal perfusate was incubated for 60 min at 37°C at a pH of 7.8 and no degradation of antipyrine or d-glucose was detected. No adsorption of either antipyrine or D-glucose to the catheters was found. Antipyrine was found to be stable in frozen perfusate and plasma (−20°C) for at least 6 months and the samples were analysed within 2-3 months after the perfusion experiment.

Analytical Methods

Two published hplc methods designed to analyze antipyrine (18, 19) were combined and modified slightly as described elsewhere (12). The accuracy for the sample concentrations 0.167, 2.09 and 10.45 μg/ml of spiked concentrations processed at the same occasion was 100.6%, 101.4% and 106%, respectively. The intra-assay variability for the same samples was 4.0, 0.7 and 0.5%, respectively. The accuracy for the control sample concentrations 0.196, 3.92 and 11.76 μg/ml which were analyzed as duplicates on different days was 99%, 100.3% and 100.9%, respectively. The inter-assay for the same samples was 7.6, 1.6, and 2.1%, respectively. The limits of quantification in plasma and intestinal perfusate were 0.1 μg/ml and 0.1 μg/ml, respectively. The analytical method was found to be linear between 0 to 15 μg/ml, which imply that all perfusate sample were diluted 200 times. The selectivity was tested from blank and spiked biological samples and no interference with degradation product and metabolites was found. Perfusate samples (0.5 g) were weighed and the total radioactivity of ^{14}C-PEG 4000 was determined by liquid scintillation counting (dpm) for 10 min (Beckman

instrument, model 244) after the addition of 8 ml Beckman Ready Safe®. The radioactivity was corrected for quenching using the internal standard of the instrument. D-glucose and the electrolytes Na^+, K^+, HCO_3^- and Cl^- were assayed using an automatic multianalyzing instrument (Hitachi 717, Boehringer Mannheim). The osmolality of the outlet perfusion solutions was measured by the vapor pressure method (Vescor osmometer 5500).

Calculations

Calculations were made from 4-5 steady state concentrations in the perfusate leaving the rectal segment. Equilibrium in the perfusate within the closed intestinal segment was considered to have been achieved when the concentrations of the solute and the ^{14}C-PEG 4000 in the outlet perfusate reached a plateau (between 60-100 min in each period). The volume of the intestinal segment (Vs) during each sampling interval was estimated using equation 1:

$$Vs = \frac{\Sigma PEG_{in} - \Sigma PEG_{out}}{[PEG]_{out}} - \text{tube volume} \quad (1)$$

where ΣPEG_{in} and ΣPEG_{out} are the accumulated amounts of ^{14}C-PEG 4000 entering and leaving the segment during the sampling period and [PEGout] is its concentration in the outlet perfusate. The mean residence time (MRT) of ^{14}C-PEG 4000 during each sampling interval was calculated by dividing the total content of ^{14}C-PEG 4000 (dpm) left in the segment by the outflow rate of ^{14}C-PEG 4000 (dpm/min). The net water flux (NWF) per cm of the isolated rectal region was calculated using equation 2:

$$\text{Net water flux} = \left(1 - \frac{([PEG]out)}{([PEG]in)}\right) \cdot \frac{Qin}{L} \quad (2)$$

where $[PEG]_{in}$ and $[PEG]_{out}$ are the entering and leaving dpm/ml of ^{14}C-PEG 4000. Q_{in} is the perfusion flow rate entering the rectal segment, which is obtained by dividing the total volume entering the segment by the sampling time (10 min). L is the length of the segment (8 cm).

The fraction disappearing from the perfusate when it has passed through the intestinal segment is assumed to be absorbed. The fraction absorbed (fa) of antipyrine was calculated from the ratio of the fluid-corrected concentrations leaving (C_{out}) and entering (C_{in}) the intestinal segment during steady-state (equation 3):

$$fa = \left(1 - \frac{(Cout \cdot [PEG]in)}{(Cin \cdot [PEG]out)}\right) \cdot 100 \quad (3)$$

The gradual increase of the outlet perfusate concentrations of PEG 4000, support our hypothesis that the solution in the isolated segment was well-mixed. This PEG 4000 concentration profile was gradually approaching the equilibrium level which was similar to that obtained by the jejunal perfusion system, that has been shown to be best described by a well-stirred model in a residence time distribution analysis (12,20, 21). Assuming a well-stirred system, the effective intestinal permeability (Pe) of the drug was calculated using equation 4 (21):

$$Pe = \frac{Qin \times (Cin - Cout)/Cout}{2\pi rL} \quad (4)$$

where $2\pi rL$ is the area of the mass transfer surface within the rectal segment that is assumed to be the cylinder area with a length (L) of 8 cm and a radius (r) of 1.75 cm.

The fraction of antipyrine absorbed into the systemic circulation calculated from plasma concentration data, F (bioavailability), was performed by deconvolution (22). The mean values of the rate constants ($\lambda_1 = 5.0\ tim^{-1}$, $\lambda_2 = 0.06\ tim^{-1}$) and the intercepts ($C_1 = 20\ \mu g/ml$, $C_2 = 17\ \mu g/ml$ and a dose of 10 mg/kg) obtained from the literature (11) were used in the weighting function.

Variability is expressed as standard deviation (SD) throughout the paper. To evaluate differences in absorption between the two experimental periods in each perfusion experiment, one-way analysis of variance (ANOVA), followed by Fisher's contrast test were used.

RESULTS

Effect of Luminal D-Glucose on the Rectal Absorption of Water and Antipyrine

The recovery of the volume marker ^{14}C-PEG 4000 was excellent and reproducible. The pH of the perfusion that entered the rectal segment was 7.4 and increased in the outlet perfusate to approximately 8.7. The osmolality was isotonic and similar during the two perfusion periods. No difference of MRT was seen between the two perfusion periods, which indicates a relative reproducable and stable residence time within the rectal segment (Table I).

With D-glucose present in the perfusion fluid the effective permeability (Pe) of antipyrine was, on average, 1.3×10^{-4} cm/s and similar when D-glucose was absent. In accordance with these results, neither fraction absorbed (fa) nor NWF was influenced by D-glucose. The individual values of NWF and Pe for antipyrine are illustrated in Figures 2A and 3A.

Effect of Rectal Transmucosal Water Flux on the Absorption of Antipyrine

The technical data, such as PEG recovery, pH, osmolality, and MRT of the periods P3 and P4 at the second perfusion occasion are given in Table I. As can be seen, the values were similar to those in the first perfusion experiment. However, the osmolality in the inlet perfusate during the fourth period (P4) was 144 ± 6.6 mOsm/l, which led to an increased net water absorption across the rectal muscosa from 0.1 ± 0.4 (P3) to −1.3 ± 1.0 (P4) ml/hr/cm ($p < 0.05$), respectively. As in experiment I, there was no absorption of D-glucose in the human rectum. The effective permeability (Pe) of antipyrine increased from 0.8 ± 0.3 (P3) to 1.9 ± 1.0 (P4) ($\times 10^{-4}$ cm/s) ($p < 0.05$) in parallel with the increased water absorption. The fraction absorbed (fa) of the drug was 12 ± 4.1 (P3) and 23 ± 9.3% (P4) ($p < 0.05$), respectively. The individual values of NWF and Pe for antipyrine are given in Figures 2B and 3B. The net flux of the electrolytes in the two periods were not to affected by the increased water absorption (Table II).

The intestinal permability of antipyrine and the net water absorption value have been estimated by a regional jeju-

Table I. Mean Values (±SD) of Technical and Absorption Parameters Obtained During a Perfusion of a Closed Human Rectal Segment of 8 cm with a Flow Rate of 3.0 ml/min

Experimental period	Perfusion occasion I		Perfusion occasion II	
	P1	P2	P3	P4
PEG rec. (%)	95 ± 10	94 ± 9	93 ± 7	106 ± 13
pH	8.6 ± 0.3	8.7 ± 0.2	8.7 ± 0.3	8.9 ± 0.1
Osmol, (mosm/l)	279 ± 8	279 ± 7	277 ± 3	144 ± 7
MRT (min)	14 ± 7	17 ± 8	11 ± 4	15 ± 4
Pe (*10-4, cm/s)	1.3 ± 0.5	1.2 ± 0.7	0.8 ± 0.3[1]	1.9 ± 1.0[1]
fa (%)	17 ± 6	17 ± 8.2	12 ± 4.1[2]	23 ± 9.3[2]
NWF (ml/h/cm)	0.7 ± 1.3[3]	0.2 ± 0.4[4]	0.1 ± 0.4[5]	−1.3 ± 1.0[3,4,5]

Abbreviations: PEGrec = recovery of the non-absorbable marker PEG 4000, MRT = residence time of the pefusion solution within the rectal segment, Pe = effective permeability of antipyrine, fa = fraction absorbed of antipyrine, NWF = net water flux across the rectal epithelium (−indicates absorption). Periods with the same number in the exponent are significantly different, $p < 0.05$.
Experimental periods P1 and P3 are control periods (isotonic and 10 mM D-glucose). During period P2 no D-glucose was present and in period P4 hypotonic condition was used.

nal perfusion technique during similar conditions (14). In Figure 4A-B a comparison between these parameters obtained in the human jejunum and rectum are presented. The Pe of antipyrine and the NWF were 5-6 and 3-4 times, respectively, higher in the jejunum than in rectum (Figure 4A-B).

The mean fractions absorbed (fa) based on perfusate concentrations of antipyrine were 17 ± 5.1 and 17 ± 5.2% for perfusion experiments I and II, respectively (Table III). An estimate of the systemic absorption (F) of antipyrine was obtained by deconvolution of the plasma concentrations of the drug. The individual F-values from all perfusion experiments are shown in Table III.

DISCUSSION

The purpose of this study was to evaluate the usefulness of a recently developed colorectal perfusion technique to investigate drug permeability and absorption in humans. The results suggest that the colorectal perfusion technique is a feasible approach to study permeability of compounds *in vivo* in humans. The results supporting this view are; (a) a high and reproducible recovery of the volume marker, (b) a stable residence time of the solution within the test segment during the perfusion experiment, (c) a net flux of electrolytes that agrees with earlier reports (7, 8, 23), (d) the solution

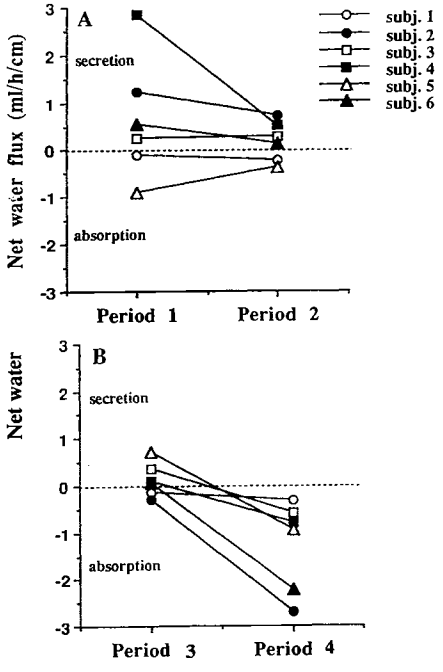

Fig. 2. The individual values of net water flux across the human rectal mucosa in the presence (P1) and absence (P2) of 10 mM D-glucose. B. The individual values of net water flux across the human rectal mucosa during isoosmolar conditions (P3) and hypoosmolar conditions (145 mOsm/l) (P4).

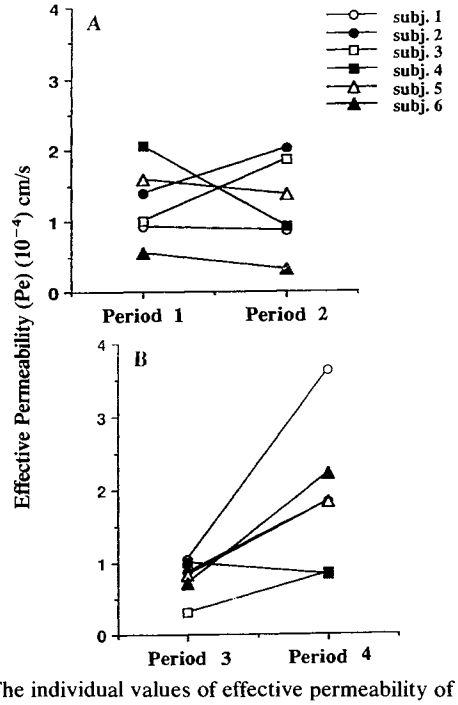

Fig. 3. The individual values of effective permeability of antipyrine across the human rectal mucosa in the presence (P1) and absence (P2) of 10 mM D-glucose. B. The individual values of effective permeability of antipyrine across the human rectal mucosa during isoosmolar conditions (P3) and hypoosmolar conditions (145 mOsm/l) (P4).

Table II. Mean Values (±SD) of the Inlet and Outlet Perfusate Concentrations (mM) of the Electrolytes Na^+ Cl^-, K^+, and HCO^{3-} Following Rectal Perfusion in Humans at a Flow Rate of 3 ml/min

	Perfusion occasion I		Perfusion occasion II	
Experimental period	P1	P2	P3	P4
Na^+ inlet (mM)	133 ± 3	134 ± 2	131 ± 4	33 ± 1
Na^+ outlet (mM)	130 ± 2	132 ± 1	130 ± 3	36 ± 2
Cl^- inlet (mM)	135 ± 6	135 ± 2	133 ± 3	48 ± 2
Cl^- outlet (mM)	129 ± 3	130 ± 2	125 ± 6	51 ± 3
K^+ inlet (mM)	5.6 ± 0.2	5.7 ± 0.1	5.6 ± 0.3	20 ± 0.5
K^+ outlet (mM)	7.7 ± 1.3	6.9 ± 0.6	7.2 ± 0.3	22 ± 1.4
HCO^{3-} inlet (mM)	0.6 ± 0.4	1.1 ± 0.4	1.1 ± 0.8	0.8 ± 0.7
HCO^{3-} outlet (mM)	5.7 ± 2.0	5.5 ± 1.8	5.1 ± 1.0	5.9 ± 1.4

within the closed test segment behaves according to a well-mixed model, (e) mass-balance absorption of antipyrine across the rectal barrier, and (f) that the procedure was well tolerated by all individuals. Furthermore, we were able to obtain a permeability value of antipyrine in rectum that was about 1.0-1.2 ($\times 10^{-4}$) cm/s. The permeability of antipyrine in the proximal jejunum in humans determined by a similar regional perfusion approach gave a Pe-value of 5.7 ± 3.5 ($\times 10^{-4}$) cm/s (14). This regional difference in passive permeability of different parts of the gastrointestinal tract can be assessed by using another in vivo technique, which measures the potential difference (PD) across the intestinal mucosa during perfusion of specially selected test solutions (23). The distal colon and rectum are parts of the gastrointestinal tract that exhibit a PD of about −35 mV, which is related to a more tight epithelium preventing the passive flux of electrolytes and water across the intestinal mucosa, mainly through the paracellular space (24). The intestinal mucosa in jejunum is considered to be more leaky, which is also reflected by the higher permeability of electrolytes and fluid and thereafter the much lower PD of approximately −3 mV (23). These permeability differences at different sites of the gastrointestinal tract obtained with different techniques, such as the PD measurements and our two regional intestinal perfusion systems, agree very well (12-14, 23). This further supports our assumption that the present perfusion technique is a feasible method to study absorption of compounds in humans.

Possible explanations of the lower Pe in the rectum compared to the jejunum for antipyrine are the tighter rectal epithelium, less fluidity in the brush-border membrane, a thicker, unstirred water layer (UWL) and a decreased mucosal surface area (23, 25, 27, 30). The tighter and less fluid rectal epithelium is probably due to a change in the lipid composition, such as increased cholesterol/phospholipid molar ratio and degree of saturation of the fatty acid residues (25). Furthermore, we also suggest that UWL is an essential factor since it might be thicker and more coherent in the colonic-recal region (30) compared with what we and others have found in the jejunum in man (12, 26). In our previous studies, we proposed that the UWL in the human jejunum does not contribute significantly to the variability in perme-

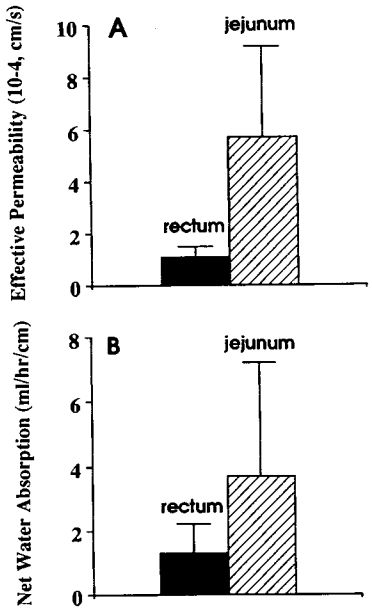

Fig. 4. The regional difference in permeability (mean ± SD) of antipyrine between rectum and jejunum in man during isotonic conditions (see ref. 14). B. Net water absorption values (mean ± SD) during hypotonic conditions in rectum (145 mOsm/l) and jejunum (175 mOsm/l) in man (see ref. 14).

Table III. Individual and Mean Values of F and fa (%) of Antipyrine Following a Rectal Perfusion in Humans During a Flow Rate of 3 ml/min

	Perfusion occasion I		Perfusion occasion II	
Subject	F (%)	fa (%)	F (%)	fa (%)
1	15	14	9.7	26
2	10	22	14	28
3	14	19	8.5	9.0
4	11	20	14	14
5	17	20	17	18
6	7.0	7.0	11	18
Mean	12	17	12	17
SD	3.3	5.1	3.0	5.2

The F-values are obtained by deconvolution of the plasma concentrations of antipyrine and fa-values are estimated from perfusate data.

ability and absorption rate for compounds with high permeability (12).

The present study demonstrated that net water absorption in the human rectum was possible to induce by perfusion with a hypotonic solution (~145 mOsm/l). The net water absorption value obtained was −1.3 + 1.0 ml/h/cm. In a previous study, we found a net water absorption value of approximately −3.7 ± 3.5 ml/h/cm in the proximal jejunum in humans, when a closed segment was perfused with a hypotonic (about 180mOsm/l), D-glucose (80 mM)-electrolyte solution (14). This is in accord with our regional difference in Pe for antipyrine in the rectum and jejunum (12-14). The lower degree of absorption of water in the rectum might be explained by a smaller pore radius, tighter epithelium, less fluidity in the rectal membrane, less number of pores in the rectal region, and a decreased mucosal surface area (23-25, 27). It has been proposed that the electrolytes and water are transported transcellularly in colon/rectum, which is different from more high permeability tissue where the electrolytes and water transport have been assumed to go by the paracellular route (24).

Earlier reports based on *in vitro* and *in situ* studies in animals have demonstrated that osmotic loads, glucose, and amino acids can lead to increased permeability in the small intestine due to an increased paracellular absorption (diffusion and/or convective flow) by a special regulating mechanism (4, 15-17). Such an approach to increase the permeability and the absorption of drugs has been considered to be a promising way to optimize drug delivery (28). In our study, the presence of D-glucose at a luminal concentration in rectum of 10 mM induced neither a net water absorption nor a permeability increase for antipyrine. The reason might be that D-glucose is not absorbed in the rectum, probably due to the absence of Na^+-nturient cotransporters, which have been proposed to trigger the dilatation of the tight junctions and increase the paracellular absorption (17). However, such a nutrient induced increase of permeability might have been obtained by luminal presence of short-chain fatty acids, which has been shown to be valid *in vitro* (29). The obtained net water absorption value (~ −1.3 ml/hr/cm) increased the permeability of antipyrine from 0.8 to 1.9 ($\times 10^{-4}$ cm/s) ($p < 0.05$). This is not in agreement with our recently results from a perfusion study of the proximal jejunum in man (14). In that report, an increased net water absorption did not result in increased permeability of the compounds antipyrine, atenolol (MW 266, $K_D = -1.8$), and enalaprilat (MW 348, $K_D = -5.2$, K_D defined as octanol/water at pH 7.4) (14). This regional difference in effect of transmucosal water flux on drug absorption is probably related to morphological factors of the rectal mucosa, such as a more tight rectal epithelium, less fluidity of the membrane, and a smaller surface are (23-25, 27). The more pronounced effect of the absorption of drugs during an increased convective flow across the barrier is in agreement with indications that different absorption enhancers seem more effective in the colon/rectum compared to the small intestine (1, 29). The mechanism(s) explaining the increased permeability of antipyrine might be due to paracellular absorption (diffusion and/or convective flow) and/or increased concentration gradient of the drug close to the rectal wall (and increased transcellular uptake).

In a recent review, Nellans emphasized the importance of investigating if an *in vitro-in vivo* correlation in drug permeability exist in the colorectal region (28). In the present study, we have shown that antipyrine has a lower permeability in rectum compared to proximal jejunum in humans (12-14), a finding that contracts with recently published results from *in vitro* animal studies of drug permeability (5, 6). In these two animal studies it was demonstrated that drugs with similar physico-chemical and biopharmaceutical factors as antipyrine, had a higher permeability in colon and rectum compared with jejunum (5,6). One plausible reason for the contradictory results between these *in vitro* studies and our *in vivo* human studies might be that the animal intestinal tissue has lost its viability, with increased absorption as a consequence. This indicates that *in vitro* methods used in absorption studies must be performed during continuous viability monitoring such as PD measurements (23).

The concentrations of Na^+ and Cl^- did not change in the outlet perfusion solution, indicating that no net transport of these two ions occurred across the rectal epithelium. However, K^+ and HCO_3^- were secreted into the rectal lumen, which is in accord with earlier reports (8, 23, 24), and support our assumptions of a normal physiological function of the investigated rectal segment. The rather high secretion of HCO_3^- might also explain the increase of perfusate pH.

The mean fractions absorbed (fa) based on perfusate concentrations of antipyrine were 17 ± 5.1 and 17 ± 5.2% for perfusion experiments I and II, respectively. The bioavailability (F) of antipyrine determined by deconvolution of the plasma concentration was on the average about 28% lower in both experiments. However, the lower mean values were mainly due to drastically lower F-values in some of the subjects (Table III). The reasons are probably because of considerable inter- and intraindividual variability in disposition of antipyrine. Despite this small discrepancy in the mean values of fa and F, we suggest that the mass balance criteria for absorption of antipyrine across the rectal epithelium is fulfilled.

In conclusion, we showed that the permeability of antipyrine and water is lower in the rectum compared with the jejunum in humans (12-14). Furthermore, a luminal concentration of D-glucose alone at 10 mM was not sufficient to induce the net water absorption across the rectal epithelium, probably due to the absence of Na^+-nutrient cotransporters. However, the present study clearly demonstrates that it was possible to increase the permeability of a small drug in the rectal region by inducing a net water absorption by lowering the osmolality. This indicates that it is possible to modulate the absorption of drugs in humans in the colorectal region. We also emphasize the importance of conducting this kind of perfusion experiments in humans with a robust method, that has been validated with respect to mass balance criteria, physiological function of the investigated intestinal part (as shown by water absorption and net flux of electrolytes), a reproducible MRT within the segment, and that the procedure was well tolerated by the subjects.

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